THE ACTION OF OESTRADIOL ON DESOXYRIBONUCLEOPROTEIN AS THE CAUSE OF ITS INHIBITORY EFFECT UPON MITOSIS IN THE SEA URCHIN EMBRYO*

by

IVAR AGRELL AND HENRY PERSSON

Zoophysiological Institute, University of Lund (Sweden)

The inhibitory effect of oestradiol upon mitosis can be seen to be accompanied in the sea urchin embryo by a dissociation of such cytoplasmic structures as the asters and spindle. The action can be observed in a concentration as low as $10^{-6}M$. Oestradiol does not seem to have its effect upon the contractibility of the elements in the asters and spindle but simply loosens the attachment between them, multipolar spindles and branched asters are formed and at anaphase the chromosomes are spread in the cytoplasm[1]. The effect is similar to the action of colchicine. Oestradiol has no demonstrable action upon the energy metabolism of the embryo, neither on respiration and glycolysis nor on the ATP-system. However, oestradiol acts upon the synthesis of the desoxyribonucleic acid (DNA) in the embryo, but has no action upon the ribonucleic acid. Under the influence of oestradiol the total amount of DNA decreases during the embryonic development in spite of the fact that the chromosomes go on multiplying to a certain extent. By the addition of DNA-metabolites, the adequate purine, and pyrimidine bases, the inhibitory effect of oestradiol can be partially eliminated. In the early sea urchin embryo nearly all the DNA is in the cytoplasm. This DNA is to a great extent consumed during the period of the early cleavages. After this time the embryo starts synthesizing DNA itself. There is a very close parallelism between the amount of cytoplasmic DNA and the sensitivity to the action of oestradiol. This sensitivity decreases with increasing age of the embryo.

The facts mentioned in this introductory part represent some results of an earlier investigation[2].

It seems very likely that the DNA of the cytoplasm is decomposed by desoxyribonuclease (DNase) and is thus made available to the dividing nucleus. Therefore we supposed that oestradiol might have an effect upon this DNase activity.

MATERIAL AND METHODS

As material for the preparation of DNA or DNA-protein, we used the sperm of the sea urchin *Paracentrotus lividus*. A native DNAP was obtained by the method of BERNSTEIN AND MAZIA[4,5]. A denaturated DNAP was prepared according to the method of WYATT[6]. A water-soluble nucleoprotein can be obtained in this way by a partial and controlled SEVAG-treatment. A pure DNA was also prepared by completing this method with further SEVAG-treatment after a preliminary

* This investigation has been carried out at Station Biologique, Roscoff, France.

References p. 546.

trypsin digestion. The crystallized DNase employed was from Worthington Biochemical Sales Co. and the oestradiol was kindly put at our disposal by N.V. Organon.

The possible action of oestradiol upon the DNase activity was tested in the following way. Cellophane sacs closed at the lower end were hung in a solution of 0.005 *M* $MgCl_2$. The sacs also contained 0.005 *M* $MgCl_2$ and DNA or desoxyribonucleoprotein (DNAP) in a concentration of about 20 γ DNA per ml. DNase in a final concentration of 20 γ per ml as well as oestradiol in a final concentration of 10 or 5 γ per ml could be pipetted into the sacs. In the latter case the surrounding solution also contained the same concentration of oestradiol. The relative amount of the fluid in the sac and the surrounding solution was 1 : 30. Air was bubbled through the system for mixing. The pH was 6.5 and the temperature +22° C. The level of the solution in the sacs was adjusted to that of the surrounding solution. By this simple arrangement the decomposition of the DNA or the DNAP could easily be followed as the decomposition products dialysed out through the membrane. Samples were taken out of the sacs at intervals and read in a Beckman spectrophotometer. For every reading the complete absorption curve between 220 and 290 mμ was drawn. Every series of experiments was represented by at least three sacs. One containing DNA or DNAP, kept as a control, one containing DAN or DNAP + DNase and one or several sacs containing DNA or DNAP + DNase + oestradiol of varied concentrations. With DNA or denaturated DNAP, the UV-absorption in the controls was always constant during the whole experiment, up to 18 hours. Also some experiments were carried out with DNA or DNAP + oestradiol only.

RESULTS

First some experiments were made with the denaturated DNAP. According to *e.g.* ALEXANDER[2], the soluble complex of DNA and histone formed by the preparation method used would be very similar to native nucleoprotein. From Fig. 1, where one out of several identical series of measurements is reproduced, it is obvious that in

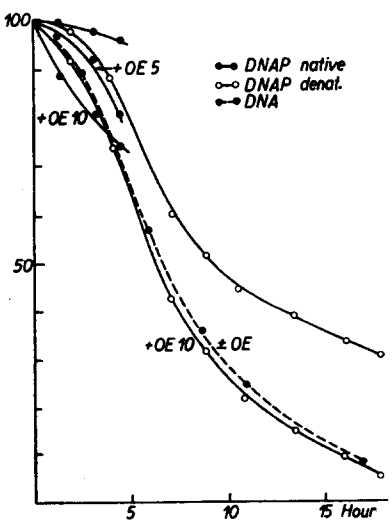

Fig. 1. The decomposition of desoxyribonucleoproteins (DNAP) and desoxyribonucleic acid (DNA) by desoxyribonuclease with or without the action of oestradiol in a concentration of 5 or 10 γ per ml. Horizontal axis: Time in hours. Vertical axis: Per cent intact DNA or DNAP.

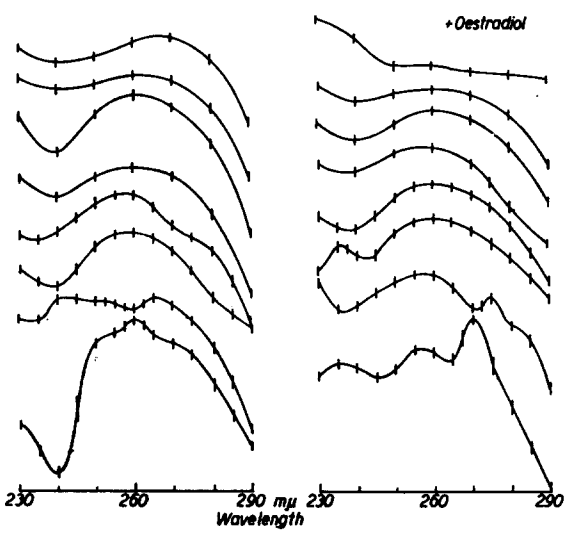

Fig. 2. The absorption curves of the decomposition products successively set free by the action of DNase on denaturated DNAP in the absence or presence of oestradiol in a concentration of 10 γ per ml. The material is the same as is represented in Fig. 1. Each curve represents the sum of products which have passed the dialysis membrane during a certain period. The curve representing the first period measured, 0–2 hour, is placed at the top and that of the last period at the bottom of the diagram. The curves are drawn in a logarithmic scale to keep the same proportionality in all and are arranged independently of their absolute values.

References p. 546.

the presence of oestradiol the decomposition of the DNAP is much accelerated. The qualitative results of one such series are represented in Fig. 2. Now the problem arose as to whether oestradiol was a DNase activator or whether possibly it acted upon the substrate. Therefore a system made of pure DNA, DNase and oestradiol was tested and the result is also reproduced in Fig. 1. In this case no activation was found. The rate of decomposition was the same whether oestradiol was added or not. Thus oestradiol is not a DNase activator. Further the rate of decomposition was just the same as that of denaturated DNAP in the presence of oestradiol. Thus the action of oestradiol upon the denaturated DNAP was maximal. In the experiments with the denaturated DNAP it was observed that in the presence of oestradiol appreciable amounts of protein were set free during the first hours of the experiment and passed out of the dialysis sac. From the spectrophotometric data these proteins were of the histone type. That oestradiol could break the linkages between DNA and histone was further confirmed by experiments made in the absence of DNase both on denaturated DNAP at $+22°$ C and on native DNAP at $+4°$ C. Histones were set free in these cases also though not in the same amounts as in the presence of DNase. Finally, some experiments were made to test the effect of oestradiol upon the DNase activity on native DNAP. In this case a 0.002 M solution of $MgCl_2$ was used. As the native DNAP is very labile and decomposes spontaneously into fragments[4], which pass through the dialysing membrane, the experiments could not be carried out over more than 4–5 hours, after which time the spontaneous decomposition almost overshadowed the action of the added DNase. This is in striking contrast to the observed stability of DNA or the denaturated DNAP. However, from Fig. 1 it is clear that oestradiol has a definite accelerating action upon the decomposition of native DNAP by DNase also. It may be of interest to note that native DNAP is considerably more resistant to DNase than the denaturated DNAP or DNA.

DISCUSSION

It has thus been shown that the proteins of DNAP exert a protective action against the enzymic breakdown by DNase and that oestradiol acts as a substrate activator for DNase by separating the proteins from DNA. On the basis of these experiments it may be possible to give an explanation of the action of oestradiol upon the mitosis of the cleaving embryo.

By the action of this hormone the decomposition of DNA, especially the cytoplasmic DNA, is much accelerated and is probably far more rapid than the possible utilization of the metabolites by the dividing nucleus. The excess of metabolites may either leak out of the cell or after further degradation some may perhaps enter the ribonucleic acid metabolism. The result will be that the embryo is eventually starved for DNA. Now the action of oestradiol upon the dividing embryos always takes place within only a few minutes. This must denote that the action of oestradiol can not only be that of a starving effect. As mentioned earlier, the more the embryos take up DNA from the cytoplasm into the nuclei and thus the more the concentration of DNA within the cytoplasm decreases during the development, the less sensitive the embryos are to the action of oestradiol. This seems to indicate that DNA may form an integral part of the cytoplasmic structure in the form of DNAP. This structure is more prominent during the earlier stages. When this structural nucleoprotein is

References p. 546.

split by oestradiol the result should be a decreased viscosity of the cytoplasm, an increased exposure of SH-groups and a dissociation of the asters and spindle, all of which have actually been established.

One further possible action of oestradiol upon the events at mitosis may be underlined. When eliminating the inhibitory effect of oestradiol through addition of purine and pyrimidine bases it was found that the embryos became extremely sensitive to different proportions of these bases once a disturbance had been created by the oestradiol action. This may denote that for a proper DNA-synthesis a very definite and orderly decomposition of the cytoplasmic DNA must take place. Also by studying the absorption curves of the decomposition products in the dialysis experiments referred to it was found that in the presence of oestradiol not only a quantitatively different but also a qualitatively different result was obtained. The products set free by DNase from DNAP seemed to be different when oestradiol was present, Fig. 2. In this case it is to be expected that the synthesis of nuclear DNA out of cytoplasmic DNA would be upset. A possibility for chromosomal disturbances is seen. Perhaps it is possible to explain in this way the carcinogenic action of oestradiol.

ACKNOWLEDGEMENTS

The investigation was aided by grants from the Scandinavian Insulin Foundation, from the Swedish Cancer Research Foundation and from the Swedish Research Council for Natural Science. The authors gratefully acknowledge these grants.

SUMMARY

The action of oestradiol upon the decomposition of desoxyribonucleoproteins (DNAP) by desoxyribonuclease (DNase) was studied by the use of a dialysis technique.

It was shown that the proteins of the DNAP exert a protective action against the enzymic breakdown by DNase and that oestradiol acts as a substrate activator for DNase by separating the proteins from DNA.

The results are utilized for giving an explanation of the inhibitory action of oestradiol upon the mitosis of the embryo. It is suggested that DNA forms an integral part of the cytoplasmic structure in the form of DNAP. This structural nucleoprotein should be split by the action of oestradiol.

REFERENCES

[1] I. AGRELL, *Nature*, 173 (1954) 172.
[2] I. AGRELL, *Compt. rend. soc. biol.*, 149 (1955) 1322.
[3] P. ALEXANDER, *Biochim. Biophys. Acta*, 10 (1953) 596.
[4] M. H. BERNSTEIN AND D. MAZIA, *Biochim. Biophys. Acta*, 10 (1953) 600.
[5] M. H. BERNSTEIN AND D. MAZIA, *Biochim. Biophys. Acta*, 11 (1953) 59.
[6] G. R. WYATT, *Biochem. J.*, 48 (1951) 584.

Received August 22nd, 1955